Section Three
Blood Toxicology

3.6 Qualitative Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.7 Liquid-Liquid Extraction Procedure for the Recovery of pKa ≥9 Drug Compounds.

3.6.7.1 BACKGROUND

This method is a general blood extraction procedure for a variety of commonly encountered basic drugs that exhibit a pKa of ≅ ≥9 along with their metabolites. With the addition of appropriate internal standard(s), this same extraction method is suitable for quantitative analysis (beyond the scope of current method). The method is based upon the principle of liquid/liquid extraction. The sample pH is adjusted with a pH 12 saturated borate buffer and extracted with n-butyl chloride. Following an optional back extraction, the extract is evaporated and reconstituted with methanol. Two internal standards are used to monitor extraction efficiency and chromatographic performance. Gas chromatography in conjunction with full scan mass spectrometry is used to confirm the presence of analytes of interest.

3.6.7.2 SCOPE

This method is a general blood extraction procedure for a variety of commonly encountered basic drugs that exhibit a pKa of ≅ ≥9 along with their metabolites.

3.6.7.3 EQUIPMENT AND SUPPLIES

3.6.7.3.1 Tube Rocker
3.6.7.3.2 Vortex Mixer
3.6.7.3.3 Evaporative concentrator equipped with nitrogen tank.
3.6.7.3.4 Laboratory centrifuge capable of 3400rpm
3.6.7.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated
3.6.7.3.6 16X100mm round bottom glass screw-top tubes
3.6.7.3.7 Screw Cap for 16mm O.D. tubes
3.6.7.3.8 GC/MS Automated Liquid Sampler (ALS) vials
3.6.7.3.9 GC/MS Vial Microinsert
3.6.7.3.10 Gas Chromatograph equipped with a Mass Selective Detector (HP 6890/5973 or better) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

3.6.7.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.6.7.4.1 Methanol (Certified ACS Grade)
3.6.7.4.2 n-Butyl chloride (Certified ACS Grade)
3.6.7.4.3 pH 12 Borate Buffer
3.6.7.4.4 100mM HCl
3.6.7.4.5 1% HCl in Methanol

3.6.7.5 QUALITY ASSURANCE MATERIAL

3.6.7.5.1 Positive Control

Positive Control can be prepared with the working solution described below and/or obtained commercially.

3.6.7.5.1.1 **Positive Control Stock Solution**

Obtain 1mg/mL stock drug standard solutions through Cerilliant, Grace, Sigma or other appropriate vendor.

3.6.7.5.1.2 **Positive Control Working Solution**

Add the designated volume of stock solution to 10mL methanol. A minimum of four compounds must be used.

Solution is stable for 6-months when stored at room temperature or 12-months when stored under refrigeration. Re-make solution when deterioration is noted.

Stock Solution (1.0mg/mL)	Volume (µL)
Amitriptyline	20
Caffeine	20
Codeine	20
Diphenhydramine	20
Lidocaine	20
Meperidine	20
Methadone	20
Nicotine	20
PCP	20
Trazodone	50
Methamphetamine	20

3.6.7.5.2 Internal Standard

3.6.7.5.2.1 **Stock Solutions**
1 mg/mL Benzphetamine
1mg/mL Papaverine

3.6.7.5.2.2 **Working Internal Standard Solution [10ng/µL]**
Add 100µL Benzphetamine and Papaverine stock solutions to 10mL volumetric ball flask. QS with methanol.

Solution is stable for three months when stored at room temperature.

3.6.7.5.3 Negative Control
Negative Whole Blood

3.6.7.6 PROCEDURE

3.6.7.6.1 Initial set-up
For each control and case sample, Label two extraction tubes and one ALS vial with microinsert.

3.6.7.6.2 Sample Preparation
The same lot of negative blood must be used for the preparation of both negative and positive spiked controls.

3.6.7.6.2.1 Prepare control sample by adding 200µL mixed working control solution to 2mL negative whole blood or pipette a 2mL sample of commercially-obtained whole blood positive control.

3.6.7.6.2.2 When the optional back extraction is used, prepare one additional positive and negative control to parallel the back extraction process.

3.6.7.6.2.3 Transfer 2mL casework samples and negative whole blood to screw top extraction tubes.

3.6.7.6.2.4 Add 20µL of internal standard mixture and vortex.

3.6.7.6.2.5 Allow sample to stand 10 minutes.

3.6.7.6.2.6 Add 2mL borate buffer (pH 12). Vortex.

3.6.7.6.3 Extraction

3.6.7.6.3.1 Pipet 4mL n-butyl chloride into each tube, cap.

3.6.7.6.3.2 Place tube on rocker for a minimum of 10 minutes.

3.6.7.6.3.3 Centrifuge 10 minutes at 3400 rpm.

3.6.7.6.3.4 Transfer the n-butyl chloride layer to second tube.

3.6.7.6.3.5 Add 50µL 1% HCl in Methanol.

3.6.7.6.3.6 Evaporate to dryness under a gentle stream of nitrogen at approximately 37ºC.
If no clean-up proceed to 3.6.7.6.5.

3.6.7.6.4 Optional Sample Clean-up

3.6.7.6.4.1 Reconstitute with 50uL of 100mM HCl.

3.6.7.6.4.2 Add 1mL of n-Butyl Chloride and vortex.

3.6.7.6.4.3 Rock for 5 minutes.

3.6.7.6.4.4 Centrifuge for 5 minutes at 3400 rpm.

3.6.7.6.4.5 Discard upper n-Butyl Chloride layer.

3.6.7.6.4.6 Add 2mL of pH 12 borate solution and vortex

3.6.7.6.4.7 Add 4 mL of n-Butyl Chloride.

3.6.7.6.4.8 Rock for 5 minutes.

3.6.7.6.4.9 Centrifuge for 5 minutes at 3400 rpm.

3.6.7.6.4.10 Transfer upper n-Butyl Chloride layer into screw-top tube.

3.6.7.6.4.11 Evaporate to dryness under a gentle stream of nitrogen at approximately 37ºC.

3.6.7.6.5 Reconstitution

3.6.7.6.5.1 Add 50uL Methanol to the residue, vortex.

3.6.7.6.5.2 Transfer extract to labeled ALS vial with microinsert.

3.6.7.6.6 Preparation for Analysis Run

3.6.7.6.6.1 Into Sequence log table, enter the sample case numbers, blanks and controls.

3.6.7.6.6.2 Load samples, standards, blank and controls into the quadrant rack as noted in the sequence table.

3.6.7.6.7 Acquisition Parameters

3.6.7.6.7.1 Refer to instrument METHOD printouts for acquisition parameters.

3.6.7.6.7.2 Current acquisition method must be stored centrally as a hard or electronic copy.

3.6.7.6.8 GC-MSD Qualitative Detection and Identification Criteria

3.6.7.6.8.1 For the identification of compounds not included in positive control, analyze appropriate non-extracted reference materials.

3.6.7.6.8.2 The presence of a drug compound is indicated if the retention time for the sample versus applicable reference material does not differ by more than ±0.2 minutes and there are no significant differences in the mass spectral data. NOTE: early eluting drugs, as well as drugs known to have similar retention times and mass spectral fragmentation patterns (e.g. phentermine and methamphetamine), may not differ from the retention time of the applicable reference material by more than ±0.1 minutes.

3.6.7.7 QUALITY ASSURANCE REQUIREMENTS

3.6.7.7.1 General

3.6.7.7.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

3.6.7.7.1.2 Refer to toxicology manual sections 5.2, 5.8, and 5.10 for quality assurance and reference material authentication requirements.

3.6.7.8 ANALYSIS DOCUMENTATION

3.6.7.8.1 Case results are to be recorded in the LIMS system.

3.6.7.8.2 Original data for controls will be compiled for each analysis run and analysis must be stored centrally in the laboratory where the analysis was performed, until archiving or destruction.

3.6.7.8.3 A copy of data for controls may be stored electronically in a central location and need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

3.6.7.9 REFERENCES

3.6.7.9.1 Procedure for High pKa Drug Analysis, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2005.

3.6.7.9.2 Procedure for Back Extraction, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2006.

3.6.7.9.3 Strong Bases Extractions - Screening SOP, Courtesy of Dr. Graham Jones, Office of the Chief Medical Examiner, Edmonton, Canada, 2003.

3.6.7.9.4 Jones, G., *Postmortem Toxicology*. pp. 98-102, *in:* Clarke's Analysis of Drugs and Poisons, 3rd Edition, Moffat, A.C, Osselton, M.D. and Widdop, B., eds., Pharmaceutical Press, 2004.

3.6.7.9.5 Hearn, W.L. and Walls, H.C. Strategies for Postmortem Toxicology Investigation. pp. 937-939. *in*: Drug Abuse Handbook, S.B. Karch, ed., CRC Press, Boca Raton, FL, 1998.

Revision History

Section Three
Blood Toxicology

3.6 Qualitative Liquid-Liquid Extraction Methods for GC/MSD Confirmation
3.6.7 Liquid-Liquid Extraction Procedure for the Recovery of pKa ≥9 Drug Compounds.

Revision #	Issue Date	History/Comments
1	04-25-2002	Original Issue in SOP format
2	05-27-2003	Updated, Clarifications
3	11-21-2006	Addition of internal standard, positive control requirements specified, extraction process restructured.
4	07-28-2008	Clarified that negative blood used to prepare positive control is the same lot as used for negative control.
5	01-16-2014	Updated storage and required components in positive control. Updated positive control requirements. Amendment to 3.6.7.8 in accordance with new LIMS system. Minor formatting changes
6	03/13/2015	Clarified the method scope and relocated procedure summary to background section. Minor formatting and grammar changes. Added tube rocker, vortex mixer, pipettors and centrifuge to supplies list. Replaced "Alltech" with "Grace" for RM vendor. Removed requirement for duplicate positive controls. Added requirement for additional negative control to parallel back extraction (if used). Consolidated quality assurance paragraphs.